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A handwritten signature in cursive script that reads 'J. Billingsley'.

JULIE BILLINGSLEY
TEAM LEADER EXAMINATION
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PROVISIONAL SPECIFICATION FOR THE INVENTION ENTITLED:

"SUBSTRATE FOR COMBINATORIAL CHEMISTRY"

This invention is described in the following statement:-

This invention relates to substrates useful for combinatorial chemistry and the manufacture of solid phase arrays on such substrates.

The invention will be generally discussed in relation to substrates for use in manufacture of DNA arrays, of the type generally known as DNA chips, on substrates particularly planar substrates but the invention is not limited to that particular application but has wider ramifications and the invention is not intended to be limited to the manufacture of such DNA chips.

In its broadest form the invention relates to substrates which can be used for spatially defined deposition of any of a wide variety of chemical substances onto the substrates. Substances may include, but are not limited to, coloured materials, dyes, polymers, catalysts, anti-wetting agents and pigments, etching chemicals, layerings and reagents for de-blocking, blocking, derivatisation and activation of solid phase chemical groups. Arrays can include deoxyribonucleic acids (DNA), peptides, peptidenucleic acids (PNA), ribonucleic acids (RNA) and other solid phase chemical arrays and arrays assembled by combinatorial chemistry.

In general the manufacture of DNA chips involves the selective and sequential addition onto a substrate, of molecular units each with a protective group which is removed when the next molecular unit is to be added. One such method of manufacturing DNA arrays uses a process known as the phosphoramidite process which uses a trityl group or derivatives of the trityl group as the protective group, termed a protecting group. The invention is not limited to this process but will be discussed with respect to it.

The phosphoramidite process is a repetitive four stage process (deprotection, coupling, capping and oxidation) for the chemical synthesis of polymers particularly sequences of DNA oligonucleotides to form portions of DNA.

In the phosphoramidite process, a portion of DNA in single stranded form is built up by the sequential addition of one of the four nucleotides (in phosphoramidite form)

being the four components which make up DNA, the A, T, G and C nucleotides. Each nucleotide has a chemically removable protecting group on it. A chemical reagent known as a de-protecting agent removes the protecting group exposing a reactive hydroxyl group and in the next stage a nucleotide (in phosphoramidite form) is coupled to the growing DNA string. The next stage is a capping step where any DNA strings which were de-protected but to which a nucleotide was not coupled are permanently capped to prevent unwanted nucleotides from adding to that molecule in later coupling steps. In the final step, oxidation of the newly formed inter-nucleotide phosphite linkage is carried out to convert the linkage to a phosphotriester.

In the manufacture of DNA arrays, a number of different sequence DNA strands are built up on a substrate to enable bio-chemical analysis to take place. In this process it is necessary to selectively de-protect various portions, terminal features, of the array and it is particularly to the requirement for this selective de-protecting that one particular embodiment of substrate of the present invention is directed.

Selective de-protecting by direct light activated chemistry or photo-removable de-protecting techniques has been developed but these are somewhat inefficient resulting in short solid phase oligodeoxynucleotides in rather large unit feature sizes of 20 to 50 microns and it is an object of this invention to provide a more efficient chemical de-protecting process.

The applicant has surprisingly found that by the use of electrically charged emulsions which include the chemical de-protecting agent in the discontinuous phase and which are selectively deposited on predefined areas of a planar or other shaped substrate under the influence of an electric field, then more accurate, localised and efficient de-protecting may be possible.

In one form therefore this may not be the only or broadest form the invention is said to reside in a substrate adapted for combinatorial chemistry, the substrate having:
a support;

a conductive layer on the support;
a dielectric layer of a material which will hold an electric charge; and
a barrier layer,

whereby static or dynamic electric charge patterns may be formed in a
5 selected array upon or in the substrate to influence the movement of charged
droplets in a medium on the substrate.

In a further form the invention is said to reside in a substrate adapted for
combinatorial chemistry, the substrate having:

10 a support;
a conductive layer on the support;
a photoconductor layer of a material which dissipates an electric charge upon
receiving incident radiation thereon; and
a barrier layer,
15 whereby static or dynamic electric charge patterns may be formed in a
selected array upon the substrate to influence the movement of charged droplets in a
medium on the substrate.

In an alternative form the invention may be said to reside in a substrate adapted for
20 manufacture of DNA arrays, the substrate having:

a support;
a conductive layer on the support;
a photoconductor layer of a material which dissipates an electric charge upon
receiving incident radiation thereon; and
25 a barrier layer,
whereby static or dynamic electric charge patterns may be formed in a
selected array upon the substrate to influence the movement of charged droplets in a
medium on the substrate;
the barrier layer comprising at least in part a chemically active material to which a
30 binder molecule can be attached, whereby a selected electric charge pattern may be
generated upon the substrate by incident radiation to cause DNA oligomers to

selectively join to selected binder molecules or to DNA oligomers already joined to a binder molecule.

In an alternative form the invention may be said to reside in a substrate adapted for manufacture of DNA arrays, the substrate having:

a support;

a conductive layer on the support;

a photoconductor layer of a material which dissipates an electric charge upon receiving incident radiation thereon; and

a barrier layer,

whereby static or dynamic electric charge patterns may be formed in a selected array upon the substrate to influence the movement of charged droplets in a medium on the substrate;

the barrier layer providing a surface to which a binder molecule can be attached.

The support may be selected from a metal, glass, ceramic, or polymeric material and the support can be either clear or opaque and either flexible or rigid.

The incident radiation may be provided from either the front or rear of the substrate.

In a preferred embodiment the support may be combined with the conductive layer.

The conductive layer may be a very thin layer and may be transparent.

The conductive layer may be vacuum-deposited onto the support.

The conductive layer may be selected from a sputtered layer of metal, indium tin oxide, or salts such as quaternary ammonium salts.

The dielectric or photoconductor layer of material which retains an induced electric charge may be an active layer and the charge on this layer may be influenced by radiation selected from infrared, visible, ultraviolet or x-ray.

The dielectric or photoconductor layer may be of a material which is adapted to have a charge pattern formed thereon on by discharging an already charged surface upon incident radiation impinging thereon. The already charged surface on the substrate
5 may be provided by a corona discharge, electron beam gun, donor roller or the like.

The dielectric layer may be glass or a polymeric resin such as Mylar (PET polyethyleneterephthalate) or the like.

- 10 Where the dielectric layer is a photoconductor the material of the layer may be selected from amorphous selenium, alloys of selenium such as selenium-tellurium, selenium-arsenic, and the like. Additionally, there can be selected as photoresponsive imaging members various organic photoconductive materials including, for example, complexes of trinitrofluorenone and polyvinylcarbazole (PVK). There are also
15 disclosed layered organic photoresponsive devices with aryl amine hole transporting molecules, and photogenerating layers, reference U.S. Pat. No. 4,265,990, the disclosure of which is totally incorporated herein by reference.

The barrier layer may be adapted to prevent reaction between the carrier liquids or
20 reagents carried in the carrier liquid and components of the dielectric or photoconductor layer. Alternatively or in addition the barrier layer may be a reactive material which allows a chemical reaction with another compound at its surface to form a derivitised or functionalised surface for subsequent reaction such as with linker molecules.

25

Alternatively the barrier layer may be intrinsically reactive.

The barrier layer may be formed from silicon dioxide, silicon nitride (Si_xN_y), titanium dioxide, Tyzor™, cross-linked or partially cross-linked epoxy novolac resins,
30 polymerised oligomers, cross-linked resins and the like.

The movement of droplets in the medium on the substrate can be towards the surface such as by electrostatic attraction, movement along the surface by a combination of electrostatic attraction and repulsion between adjacent charge patterns on the surface or to enable a chemical reaction with another droplet of a chemical being moved together from a different position on the substrate. Alternatively two different types of droplets in a medium may be brought together for reaction on a charge patterned surface

Where the substrate according to the invention includes a photoconductor layer it is preferable that the photoconductor material is one that has a low or very low dark decay. Dark decay is the property of a photoconductor to dissipate an electrostatic charge before exposure to light or before the latent electrostatic image is used for whatever purpose. Examples of suitable photoconductor materials include ZnO resin dye combinations coated on metalised Mylar™ (PET polyethyleneterephthalate), vacuum evaporated cadmium sulphide on stainless steel, vacuum evaporated selenium on stainless steel belts, manufactured by Omnitek of the US, pure selenium, organic PVK photoconductor doped with trinitrofluorenone (TNF) ex Kalle, Germany.

Other suitable photoconductor materials may include lead sulphide, lead selenide, amorphous selenium, amorphous silicon, doped selenium and organic photoconductors.

The use of the substrate of the present invention for the manufacture of DNA arrays will now be discussed.

The substrate of the present invention may be used for a range of solid phase chemical reactions where it is desired to selectively spatially react on the substrate.

In one embodiment the substrate may be used in a method of forming a solid phase chemical array on the substrate using a stepwise reaction process, the method including the steps of:

- (a) defining at least one region on the substrate by forming an electrostatic charge on that region which is different from the electrostatic charge on other regions of the substrate such as by formation of a latent electrostatic image thereon,
- (b) applying an emulsion to the substrate, the emulsion having the electrically charged discontinuous phase droplets and a chemical reagent carried in or comprising the discontinuous phase,
- (c) attracting the discontinuous phase of the emulsion to the at least one preselected region by attraction by the latent electrostatic charge on the region and optionally by the use of a background bias voltage to reduce deposition in non-required regions,
- (d) causing a chemical reaction in the at least one region,
- (e) removing the emulsion, and
- (f) carrying out subsequent steps of the stepwise reaction process.

In an alternative embodiment it may be used in a method of forming a DNA array on the substrate using a stepwise coupling process with a chemical de-protecting step prior to each coupling step, the method including the steps of:

- (g) preparing a substrate with surface functional groups protected by a removable protecting group;
- (h) defining at least one region on the substrate by forming an electric field on that region which is different from the electric field on other regions of the substrate such as by formation of a latent electrostatic image thereon,
- (i) applying an emulsion to the substrate, the emulsion having the electrically charged discontinuous phase droplets and a chemical de-protecting reagent carried in the discontinuous phase as discussed above,
- (j) attracting the discontinuous phase of the emulsion to the at least one preselected region by attraction by the electric field on the region and optionally by the use of a background bias voltage to reduce deposition in non-required regions,
- (k) causing chemical de-protecting in the at least one region,
- (l) removing the emulsion, and
- (m) carrying out subsequent steps of the stepwise coupling process.

The subsequent steps of the stepwise coupling process may be such as those that are carried out in the standard phosphoramidite chemistry for synthesis of oligodeoxynucleotides although as discussed earlier the invention is not limited to this particular chemistry.

It will be realised that the process as discussed above may be repeated a sufficient number of times to synthesise selected oligonucleotides of any sequence and length up to at least 100-mer in a predetermined spatial order, position and feature size on the substrate.

The substrate may be in part insulative or dielectric material which is able to hold an electrostatic charge for sufficient time for attracting the discontinuous phase of the emulsion to the at least one preselected region. The substrate may include dielectrics such as glass, plastics materials or the like and alternatively may include photoconductors such a zinc oxide, selenium and the like.

The step of defining at least one region on the substrate by forming a latent electrostatic charge on that region may include the step of image reversal to cause deposition in non-charged regions.

The formation of the electrostatic image pattern may be by electrostatic means such as wherein the substrate is a photoconductor and the formation of the electrostatic field is by charging and subsequent discharging by selective illumination. Preferably the illumination may not include radiation in the short ultraviolet region as this may cause damage to the DNA molecule. For the assembly of other chemical chips or arrays, however, UV radiation may be used.

In one preferred embodiment, the stepwise coupling process is the phosphoramidite process which uses chemical de-protecting of a trityl group but the invention is not so limited but may include other stepwise coupling or addition processes.

The step of removing the emulsion may include the step of neutralising any residual chemical de-protecting agent in the emulsion to prevent it from reacting in non-desired parts of the array.

- 5 As discussed above, the emulsion for use for the present invention comprises an electrically insulative continuous phase such as a fluorochemical, an aqueous or a non-aqueous discontinuous phase for instance a hydrocarbon oil which carries the chemical de-protecting agent in it in solution, with preferably a surfactant and preferably a charge control agent.

10

Where the discontinuous phase is water the chemical de-protecting agent may be a weak organic acid such as acetic acid. Acetic acid may be present in a concentration of up to 80% (v/v).

- 15 Where the discontinuous phase is a hydrocarbon oil, the chemical de-protecting agent may be a strong protic organic or inorganic acid.

- The non-aqueous discontinuous phase which carries the chemical de-protecting agent in solution may be selected from acetone, acetonitrile, cyclohexanone, 20 dibromomethane, dichloromethane (methylene chloride, DCM), trichloromethane, dimethyl formamide (DMF), dioxane, 1,2 dichloroethane (DCE), nitromethane, tetrahydrofuran, toluene, dimethyl formamide or mixtures of compounds such as isopropanol/methylene chloride, nitromethane/methanol, nitromethane/isopropanol, trichloromethane/methanol or isopropanol/methylene 25 chloride. Other hydrocarbons such as decalin may also be used.

- The chemical de-protecting agent may be a Lewis acid or a protonic acid. The Lewis acid may be selected from but not restricted to zinc bromide, titanium tetrachloride, and ceric ammonium nitrate while dilute protonic acids which can be used include, 30 but are not limited to, dilute mineral acids, trichloroacetic acid (TCA), dichloroacetic acid (DCA), benzenesulphonic acid, trifluoroacetic acid (TFA), difluoroacetic acid,

perchloric acid, orthophosphoric acid and toluenesulphonic acid. Other acids may include dodecylbenzene sulphonic acid and diphenyl acid phosphate.

5 This then generally describes the invention but to assist with understanding, reference will now be made to examples of emulsions according to the present invention and discussion in more detail of the stages of formation of a DNA array.

EXAMPLE OF SPATIALLY SELECTIVE DEPOSITION OF A CHARGED EMULSION ONTO A SUBSTRATE

10 An experiment was carried out to determine whether a charged emulsion would deposit on a substrate with an electrostatic image pattern formed thereon. For this purpose an emulsion was formed with an insulative continuous phase and a discontinuous phase which included a dye.

15 For this purpose an emulsion was formed with an insulative continuous phase and a discontinuous phase which included an acid and a substrate comprising a zinc oxide photoconductor was charged with a negative pattern. The photoconductor was dip-coated with a solution of Butvar 72 (1% w/v) and the pH indicator methyl orange (at saturation) in cyclohexanone, and dried at 55 degrees C for 30 minutes:

20

The emulsion comprised the following:

Continuous phase	FC40	0.89ml
Discontinuous phase	5% dichloroacetic acid (w/v) in toluene	0.1ml
	F6H14 (1% in FC40, v/v)	0.01ml

25

F6H14 is a semifluorinated alkylalkane, 1-(perfluoro-n-hexyl) tetradecane, manufactured by Apollo Scientific Ltd, UK.

30 It was found that the discontinuous phase deposited only in the area of the charge pattern, giving a pH-dependent colour change from yellow to pink.

This then generally describes the invention but to assist with understanding reference will now be made to the accompanying drawings which show preferred embodiments of the invention and the use of the invention.

5 In the drawings:

Figure 1 shows a first embodiment of a substrate according to the present invention;
Figure 2 shows an alternative embodiment of a substrate according to the present invention;

Figure 3 shows one method by which a substrate according to the present invention may be charged for the deposition of chemicals using an emulsion; and

Figure 4 shows an alternative method by which a substrate according to the present invention may be charged for the deposition of chemicals using an emulsion.

Figure 1 shows an embodiment of the present invention in which the substrate has a support 1 to provide integrity to the substrate. On the support 1 there is a conductive layer 2. The conductive layer 2 may be a metal or other conductive material such as indium tin oxide (ITO). Where the support is itself a conductor then the conductive layer need not be present. On the conductive layer 3 there is a dielectric layer 3 which is capable of holding an electrostatic pattern image thereon. For instance the dielectric layer 3 may be a photoconductor. On the dielectric layer 3 there is in this embodiment a barrier layer 4. The barrier layer is provided to protect the dielectric layer 3 and its outer surface5 can provide a reactive surface for reactions for which the substrate is used.

Figure 2 shows an alternative embodiment of the present invention in which the substrate has a support 1 to provide integrity to the substrate. On the support 1 there is a conductive layer 2. The conductive layer 2 may be a metal or other conductive material such as indium tin oxide (ITO). Where the support is itself a conductor then the conductive layer need not be present. On the conductive layer 3 there is a dielectric layer 3 which is capable of holding an electrostatic pattern image thereon. For instance the dielectric layer 3 may be a photoconductor. The outer surface of the dielectric layer 3 can directly provide a reactive layer for reactions for which the

substrate is used. This arrangement is useful where the dielectric layer 3 is not susceptible to attack by the materials of the emulsion used for chemical or physical reactions on the surface.

5 Figure 3 shows one method by which a substrate according to the present invention may be charged for the deposition of chemicals using an emulsion. In this arrangement the substrate is charged with a single point corona discharge device to give a uniform charge pattern on the substrate. A mask is then held over or placed
10 onto the substrate and a light shone onto the mask. Where there are transparent portions in the mask the photoconductor becomes conducting and the charge pattern in those areas is conducted to the conductive layer. This leaves an electrostatic charge pattern in the unexposed areas and it is to these areas that the droplets of the discontinuous phase are attracted.

15 Figure 4 shows an alternative method by which a substrate according to the present invention may be charged for the deposition of chemicals using an emulsion. In this arrangement a metal or other conducting mask is held over or placed onto the substrate and then the substrate is charged with a single point corona discharge device. This gives a charge pattern on the dielectric layer of the substrate in those
20 areas in which there are apertures in the mask areas and it is to these areas that the droplets of the discontinuous phase are attracted.

While this solid phase combinatorial chemistry synthesis process has been discussed in relation to the phosphoramidite process it is to be realised that the process is also
25 applicable to other processes which use a stepwise addition process with a chemical protection or de-protecting step or a chemical activation or deactivation step, derivatisation step or coupling step on a substrate. Indeed it is possible that the activated amidites could be selectively electrostatically deposited onto a completely de-protected substrate.

30 Throughout this specification various indications have been given as to the scope of this invention but the invention is not limited to any one of these but may reside in

two or more of these combined together. The examples are given for illustration only and not for limitation.

Throughout this specification and the claims that follow unless the context requires otherwise, the words 'comprise' and 'include' and variations such as 'comprising' and 'including' will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Dated this 30th day of June 2003.

RAUSTECH PTY LTD
By its Patent Attorneys
MADDERNS



Figure 1 - with Barrier layer

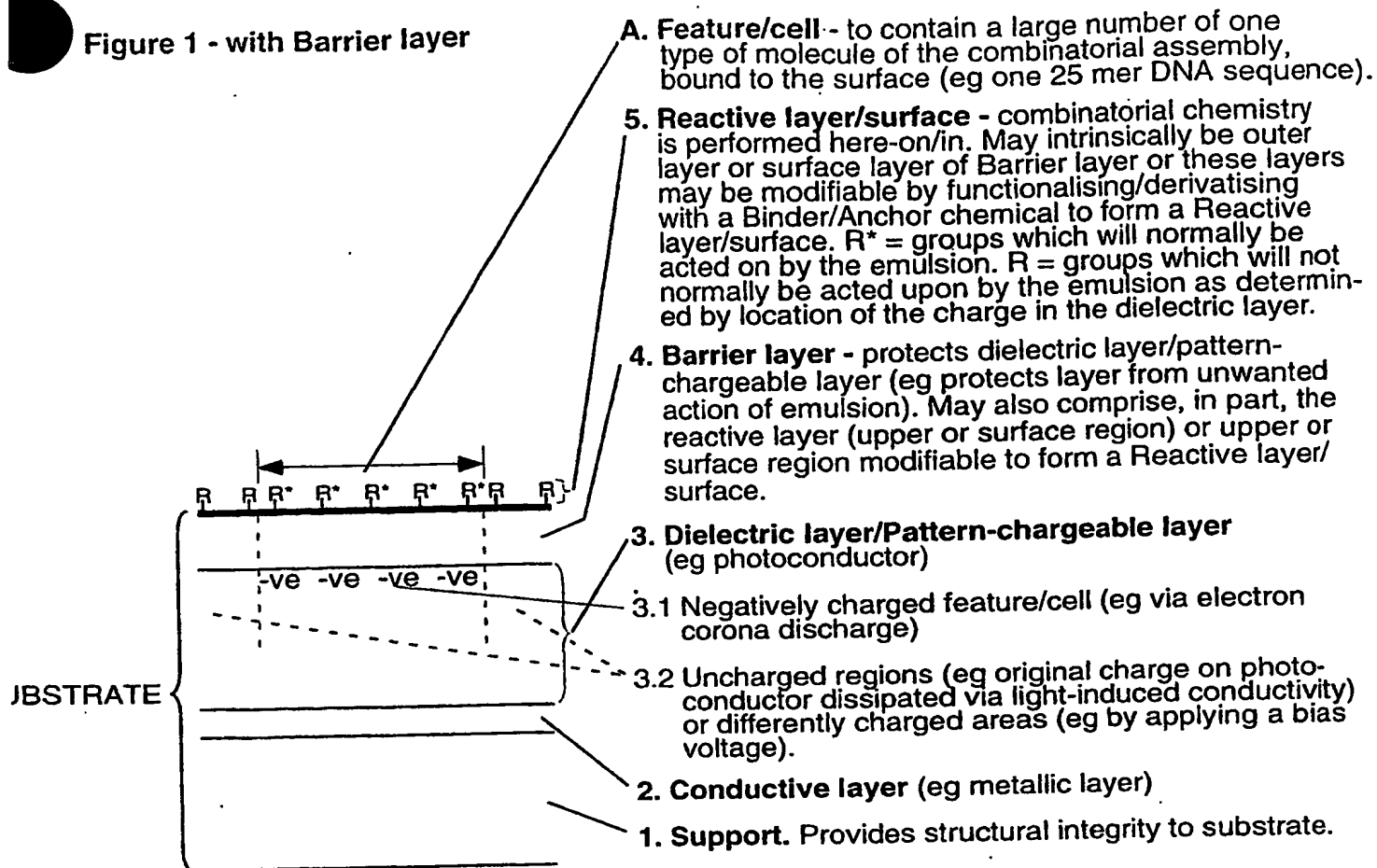


Fig 1

Figure 2 - without Barrier layer
See Fig 1 for further details

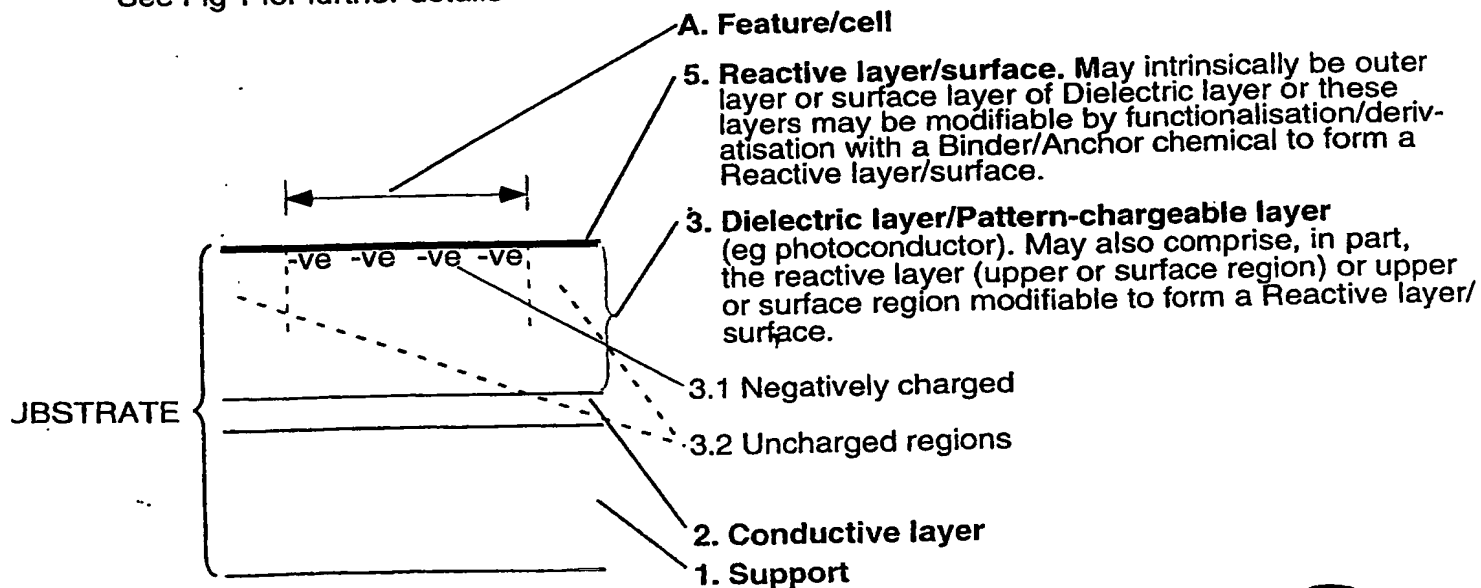


Fig 2

Fluorophor deposition of a mask replica image on a photoconductor strip. Features comprising a mask replica in charged form were generated by selective light directed discharging through a mask of a completely electron-corona-charged photoconductor, with the fluorophor subsequently deposited thereon via electrostatic attraction of oppositely charged fluorophor-bearing emulsion droplets.

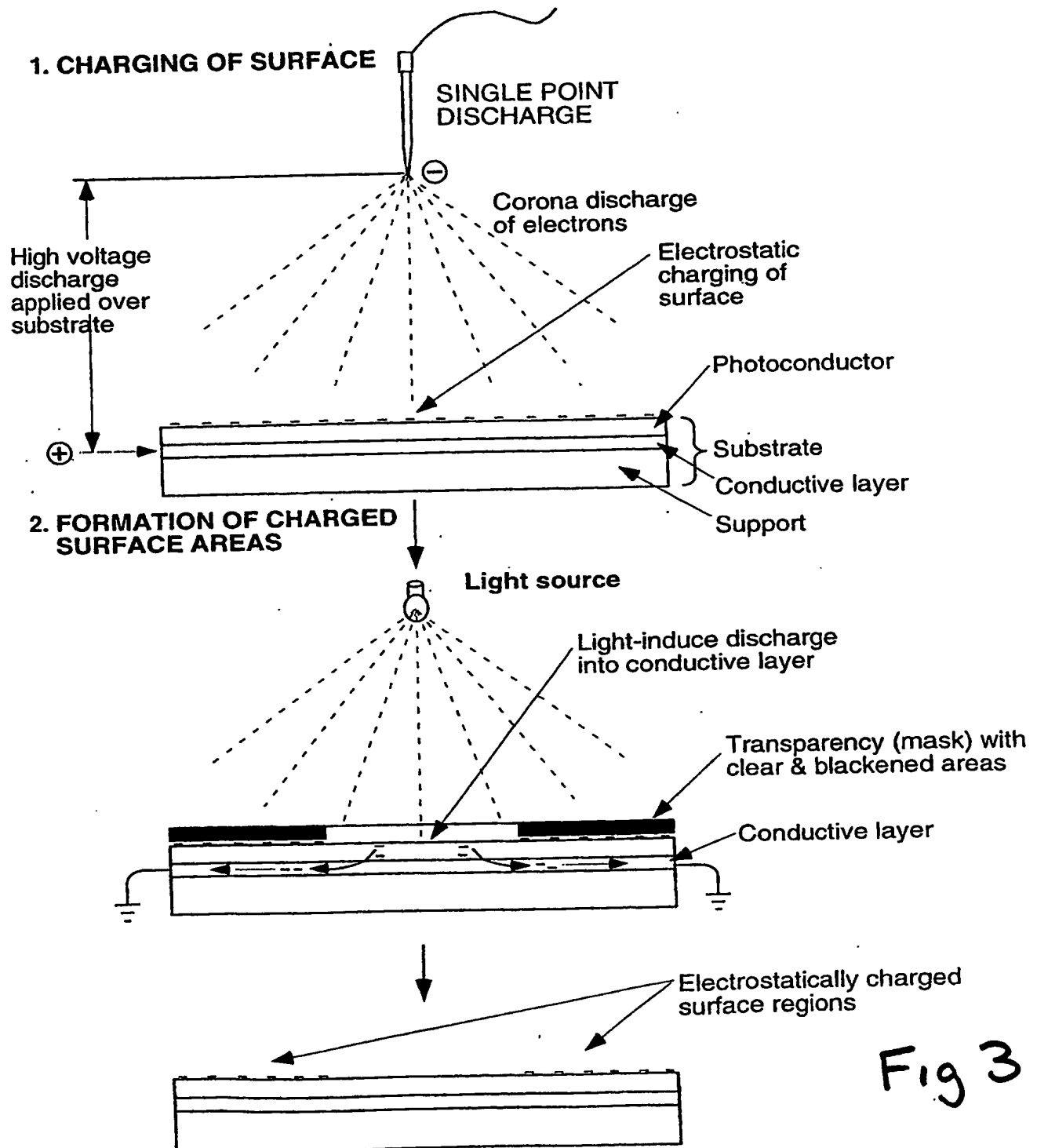


Fig 3

Fluorophor deposition of a mask replica image on a photoconductor strip. Features comprising a mask replica in charged form were generated by selective light directed discharging through a mask of a completely electron-corona-charged photoconductor, with the fluorophor subsequently deposited thereon via electrostatic attraction of oppositely charged fluorophor-bearing emulsion droplets.

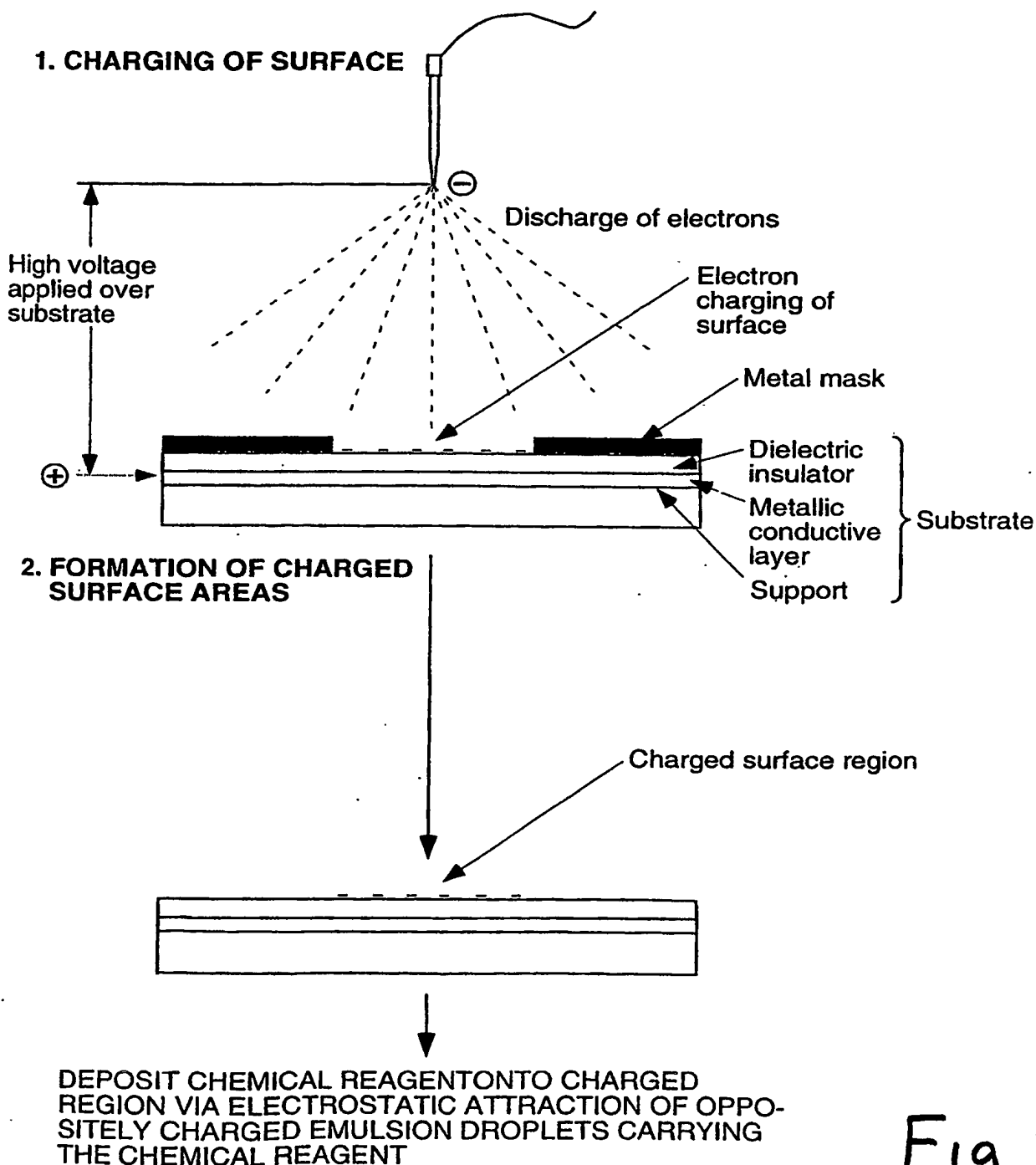


Fig 4

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